PHARMACOKINETICS OF INHALED 2-NITROPROPANE IN THE RAT - B. Denk, E. Deml, D. Oesterle, K.H. Summer and J.G. Filser, Institut für Toxikologie, GSF, D-8042 Neuherberg, FRG

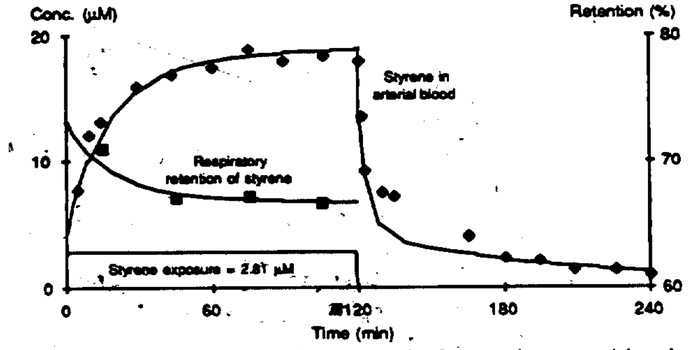
The solvent 2-nitropropane (2-NP) is referred to dose-dependently produce liver necrosis and hepatocarcinoma in the rat. Since this may be caused by reactive metabolites we studied the rate of metabolism in dependency of the atmospheric concentration of 2-NP.
Two male or two female Sprague-Dawley rats were exposed simultaneously to gaseous 2-NP in closed all glass exposure systems (21 l). Due to extensive reaction of 2-NP with the carbon dioxide absorbent soda lime the latter was omitted. The initial gas concentrations ranged from 50 ppm up to 1600 ppm. The concentrations were measured gaschromatographically. Concentration-time-curves were followed over the exposure time up to 3 h. In two experiments the experimental procedure was modified to allow 8 h exposure. Pharmacokinetic parameters were determined as previously described [Simon et al. (1985), Arch. Toxicol. 57, 191].
At all concentrations no saturation kinetics were observed. In both sexes the decay of 2-NP followed strictly first order kinetics. The metabolic clearance correlated with the atmospheric concentration and amounted to 24000 ± 3000 (ml/h·kg b.w., ± S.D.). This value approaches the ventilation rate in this species. The rate of metabolism was directly proportional to the exposure concentration and reached 980 umol/h·kg b.w. at 1000 ppm. From the linear kinetics and the high metabolic clearance it can be deduced that the rate of 2-NP metabolism is mainly limited by pulmonary uptake.
Additionally, at exposure concentrations of up to 100 ppm pretreatment with dithiocarb (200 mg/kg b.w., i.p.), an effective inhibitor of cytochrome P450 mediated metabolism, resulted in an inhibition of 2-NP metabolism by only 60%. This supports in vitro findings that 2-NP metabolism not exclusively proceeds via mixed function oxidases.

PHYSIOLOGICALLY BASED MODELING OF ORGANIC SOLVENT TOXICOKINETICS BY SPREAD-SHEET PROGRAMMING ON A PC - G. Johanson and P.H. Näslund, National Board of Occupational Safety and Health, S-171 84 Solna, SWEDEN.

The principles and advantages of physiologically based kinetic modeling of xenobiotica as well as of inhaled solvents are well established. Many different models and programs have been used, but most are difficult to operate and/or limited in their application. We have developed a concept, where a simple program is run in the form of a spread-sheet program (Microsoft® Excel) macro instruction on a personal computer (Apple Macintosh™ Plus). The model parameters are entered and stored in tabular form, and any variables may be plotted. All parameters and procedures have default values and simulations can be performed by a beginner after a few instructions. With some knowledge of the Excel spread-sheet program and of physiological modeling by defining a set of mass-balance equations, the model can easily be altered or designed in any fashion, e.g. by introducing Michaelis-Menten kinetics, metabolite(s) kinetics etc.

The outcome of the Excel program was identical to that of a Fortran based program intended for the general solution of differential equations (Dare-p), when the same models were run. In terms of processing time, the Dare-p program was somewhat faster. However, considering the total time spent at the computer, the Excel program was much faster.

The concept is illustrated by program listings and graphical results from the simulation of the toxicokinetics of some organic solvents (see example below), although it is applicable for the modeling of the kinetics of any substance in the body. When using this concept the efforts may be concentrated on the problems related to the toxicokinetics of the substance, rather than on the computer and its programming.



Exposure to styrene during light physical exercise on a bicycle ergometer. Comparizon between simulated curve and experimentally obtained values. It took less than 5 min to obtain the plot, counted from the start of the simulation.

PULMONARY DEPOSITION, RETENTION, AND CLEARANCE OF A PIGMENTED POLYMER IN RATS

H. Muhle[1], B. Bellmann[1], O. Creutzenberg[1], R. Kilpper[2], and R. Mermelstein[2]

1. Fraunhofer-Institut fur Toxikologie and Aerosolforschung, Hannover 61, F.R.G.

2. Joseph C. Wilson Center for Technology, Xerox Corporation, Rochester, NY, 14644, U.S.A.

We have defined the Maximum Functionally Tolerated Dose (MFTD) of a material as the lung burden for which macrophage mediated lung clearance is not significantly impaired. In order to establish its value for the pigmented polymer, a 90-day subchronic inhalation study of a toner fraction was conducted by exposure of groups of F-344 rats for six hours/day, five days/week for 13 weeks. The test material used was a specially prepared and characterized powder sample identical to 9000 type xerographic toner, except that its ACGIH respirable fraction was enriched about 10-fold to 35%. The exposure concentrations used were 0, 1.0, 4.0, 16.0, and 64.0 mg/m^3 total mass.

Clearance results for the test material and a superimposed spike of Fe^{59} iron oxide were essentially unchanged at exposure concentrations of 0, 1 and 4 mg/m^3 when measured at different times during the exposure period. At 16 mg/m^3, some indications of slightly retarded iron oxide clearance were noted after 90 days of exposure. At 64 mg/m^3, no appreciable toner clearance was observed after 60 and 90 days of exposure, and clearance of the iron oxide tracer was significantly retarded after 30, 60 and 90 days.

Based upon the above observations, as well as the increase in lung weight, the maximum functionally tolerated dose of test material (MFTD) in this subchronic study was exceeded at the 64 mg/m^3 exposure level.

KINETICS OF THE ACCUMULATION IN THE LUNG AND ASSOCIATED LYMPH NODES OF INHALED MINERAL DUSTS DURING PROLONGED CHRONIC EXPOSURE

J.H. Vincent, A.D. Jones, A.M. Johnston, C. McMillan.
(Institute of Occupational Medicine, Edinburgh, Scotland, United Kingdom).

Experiments have been carried out using laboratory rats exposed to fibrous and non-fibrous, toxic and non-toxic, and relatively-insoluble mineral dusts under chronic exposure conditions for airborne respirable concentrations from 0.1 to 100 mg/m^3. The aim was to investigate the accumulation of dust in the lung and associated lymph nodes. The results have shown consistently that, after an initial non-linear phase during the earlier part of the exposure history, lung burden becomes a linearly-increasing function of exposure time. Furthermore, lung burden scales directly with respect to the respirable concentration over its full range. The corresponding results for the lung-associated lymph nodes suggest that substantial accumulation of dust in them does not begin until lung burden itself has reached a certain threshold. That threshold is lower for toxic than for non-toxic dust. However, once accumulation has begun, the actual rate of transport is not markedly dependent on toxicity.

The experimental results form the basis of an improved mathematical model for the kinetics of accumulation of lung and lymph node burden. The central feature of the model is the sequestration of particles at locations from which they cannot be cleared (possibly by focal aggregation in macrophages in alveolar spaces, entrapment within epithelial cells and in the alveolar interstitium and/or entrapment within areas of fibrotic pathological change).

This model is now being used to explore the relationship between exposure and dose for humans chronically-exposed to mineral dusts in the occupational environment. It provides results which are markedly different from those obtained using previous models.

CARBON FIBRES: RESULTS OF A FURTHER SURVEY OF PROCESS WORKERS AND THEIR ENVIRONMENT IN A FACTORY PRODUCING CONTINUOUS FILAMENT - H.D. Jones, Courtaulds plc, P.O. Box 16, Coventry, W. Midlands, U.K.

Carbon fibre production was started in 1972. Process workers engaged in all aspects of production have been kept under medical surveillance since the initial start-up, which included radiographic, spirometric and respiratory questionnaire and examination.

Dust concentrations have been low, mean level being 0.39mgm^{-3} total dust and 0.16mgm^{-3} respirable dust, the mean carbon fibre diameter being 8-10μ, fracturing laterally, but not longitudinally into fibrils.

Follow-up results since inception of the producing have revealed no evidence of ill effect on the lungs.

NON-LINEAR RELATIONSHIP BETWEEN THE DOSE DEPOSITED IN THE RESPIRATORY TRACT AND THE DOSE TO TARGET TISSUES. M.A. Medinsky and R.O. McClellan, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87185

Polycyclic aromatic hydrocarbons such as the respiratory tract carcinogen, benzo(a)pyrene are emitted into the atmosphere as a result of fossil fuel, gasoline and diesel fuel combustion. These compounds may pose a health risk to people if inhaled and retained in the respiratory tract. Studies were conducted in which rats were given doses of ^{14}C-benzo(a)pyrene, ranging from 6400 ng per g lung to 16 ng per g lung. Rats were sacrificed at various times after administration and the total ^{14}C in the lung and ^{14}C covalently bound to macromolecules was determined. Two-component negative exponential functions were fit to the data obtained. With increasing dose (16-6400 ng), an increasing percent (89-99.76) cleared with a short half-time (less than 1 day) and a decreasing percent (11-0.24) cleared with a half-time greater than 1 day. Equilibrium levels of benzo(a)pyrene in lungs, predicted for continuous exposure to benzo(a)pyrene, ranged from 3.3 to 100 ng/lung (a factor of 30), although the amount of benzo(a)pyrene administered varied by a factor of 400. At 24 hours after dosing, from 1 to pmoles of ^{14}C was found bound to lung macromolecules regardless of the dose administered. These data suggest that linear extrapolation from high dose studies to environmentally relevant concentrations of inhaled materials would tend to underestimate the lung burdens of benzo(a)pyrene and thus, potential health risks. [Research supported by the Office of Health and Environmental Research, U.S. Department of Energy under Contract No. DE-AC04-76EV01013.]

TOXICOKINETICS OF INHALED PURE AND PARTICLE-ASSOCIATED ORGANIC CHEMICALS. J.A. Bond, J.D. Sun, M.A. Medinsky, C.E. Mitchell, R.K. Wolff, and R.O. McClellan, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM-87185

Classes of organic compounds such as polycyclic aromatic hydrocarbons (PAH), nitro-PAH, aromatic amines, and azaarenes have been detected in emissions associated with energy production and use. These chemicals are associated with respirable particles and many have known mutagenic, carcinogenic, and/or toxic properties. There is a paucity of data on the fate, target organs, and potential human health risks associated with inhalation exposure of these chemicals. Toxicokinetic data have been obtained for inhaled benzo[a]pyrene, nitropyrene, aminoanthracene, and phenanthridone both in pure forms and associated with carbonaceous, organic, and inorganic particles. Rats were exposed by nose-only inhalation to aerosols of the different chemicals. Following each exposure, rats were sacrificed at various times and tissues were removed for analysis of parent compound and metabolites. In all cases, the rate of clearance of material from the respiratory tract occurred in two phases, an initial rapid phase and a subsequent slower phase. In general, when the chemical was inhaled in pure form, over 99% of the inhaled material was rapidly cleared with a half-time of less than 1 day. However, when the chemical was inhaled on particulate matter a significant fraction of the inhaled chemical was retained in the lungs (up to 50%) and this fraction cleared with a half-time of > 1 day (i.e. 1-30 days). The data obtained for the different chemicals have been used to predict lung concentrations of the inhaled chemicals in humans. Equilibrium lung burdens were predicted for humans using the animal toxicokinetic data and hypothetical exposure conditions (3.5 ng particles/L air, 8 hr/day, 0.1% PAH coating). The rate constants used for each calculation were taken from the toxicokinetic studies. The data indicate that chemicals coated on particless yield equilibrium lung burdens up to 1000 times higher than from exposure to pure aerosols of PAH. In summary, the results from these series of studies have shown that particle association of chemicals influences the rate of clearance of those chemicals. The data predict that under some conditions (e.g. particle association) lungs can accumulate significant quantities of inhaled chemicals. [Research performed under DOE Contract No. DE-AC04-76EV01013.]

SIGNIFICANCE OF THE EXTRAALVEOLAR PERIVASCULAR SHEATH IN THE ALVEOLAR CLEARANCE OF INSOLUBLE PARTICLES - S. Takenaka, H. Muhle, B. Bellmann and U. Mohr, Fraunhofer Institut fuer Toxikologie und Aerosolforschung, Hannover, FRG.

Previous inhalation studies on the localization of insoluble particles in the alveolar regions showed frequent deposition of the particles in the perivascular space of small blood vessels. It was suggested that the particles were at least in part translocated to the lung-associated lymph nodes after having entered the extraalveolar perivascular sheath.

In this experiment we compared the alveolar distribution of 5 types of insoluble particles inhaled at various concentrations by rats. (Fly ash from coal fired power plants, TiO2-anatase, TiO2-rutil, plastic powder, iron powder; 1 to 20 mg/m3, 3-12 months exposure).

We found frequent deposition of particles in the extraalveolar perivascular sheath in every group exposed to concentrations higher than 5 mg/m3. In addition, particle-laden alveolar macrophages had sometimes aggregated in the alveolar lumen, especially near the perivascular space and subpleural regions. At concentrations lower than 2 mg/m3, particle deposition rarely occurred in the perivascular space. However, slight accumulation of particles was always observed in the peribronchial lymphatic tissues and the lung-associated lymph nodes.

These findings confirm that the extraalveolar perivascular sheath plays an important role in the alveolar clearance of insoluble particles inhaled at high concentrations. At lower concentrations it is difficult to obtain morphological evidence for the alveolar clearance route, however, it cannot be excluded that the particles are also cleared via the perivascular space to the lung-associated lymph nodes.

ACCUMULATION OF ETHOXYACETIC ACID DURING REPEATED EXPOSURES TO THE ETHYL ETHERS OF ETHYLENE GLYCOL AND ETHYLENE GLYCOL ACETATE. - H. Veulemans, D. Groeseneken, R. Masschelein, E. Van Vlem, Department of Occupational Medicine, Cath. University of Louvain, B-3000 Louvain, Belgium.

Ethoxyacetic acid (EAA) was found to be a major urinary metabolite of both the ethyl ether of ethylene glycol (EGEE) and of ethylene glycol acetate (EGEE-Ac) in short-term experimental exposures of humans. Because of its relatively long biological half-life (22-25 h) in these experiments, it was believed that this metabolite could accumulate during repeated exposures. This was investigated in a group of 5 women with daily occupational exposure to EGEE and EGEE-Ac.
Individual exposures were monitored per shift-halves and urine samples were taken before and after shifts during 2 observation periods of resp. 5 and 7 days. The daily exposures were fairly constant with an overall mean value of 14 mg/m^3 (sum of EGEE and EGEE-Ac in equivalent amounts of EGEE). Urinary EAA excretions showed a clear increase during the work week. Elimination during the weekends was far from complete and even after a prolonged period without exposures (12 days), traces of the metabolite were still detectable in urine (1.2 - 2.6 mg/g creatinine). Maxima at the end of a complete working week were estimated at 150 $\pm$ 35 mg EAA/g creatinine for average full-shift exposures to 19 mg/m^3 EGEE or 27 mg/m^3 EGEE-Ac.

INTERSPECIES COMPARISONS OF PULMONARY RESPONSES TO INHALED PARTICLES AND FIBERS: IMPLICATIONS FOR TOXICOLOGIC EVALUATIONS - DB Warheit, MS Stefaniak, and MA Hartsky, Du Pont - Haskell Lab., Newark, DE, USA.

Some of the major challenges for inhalation toxicologists involve the extrapolation of animal data to humans and to define the mechanisms of particle-induced lung toxicity. This is a difficult task, in part because interspecies differences abound, both in the lung as well as in other organs. Since toxicologic testing frequently requires utilization of several species, it would be useful to make interspecies comparisons of inhaled particulates under identical experimental conditions. Accordingly, we are investigating the lung responses of different rodent species to standard inhaled doses of either asbestos or carbonyl iron (CI) particles. Additionally, in vitro morphologic and functional studies were carried out on pulmonary macrophages (PM) recovered by bronchoalveolar lavage to complement the in vivo inhalation studies.

Rats and mice were exposed to aerosols of chrysotile asbestos for 1-3 hr (10 mg/m^3). In addition, 2 strains of rats, 3 strains of mice, and 1 strain each of hamsters and guinea pigs were exposed to aerosolized carbonyl iron particles for 1,3, or 6 hrs at 100 mg/m^3. Scanning electron microscopy of dissected lung tissue revealed that asbestos fibers and carbonyl iron particles deposited preferentially on alveolar duct bifurcations regardless of the species. Decreased numbers of particles were counted on bifurcations of guinea pigs compared to rats and mice. Time course studies showed that increased numbers of rat PM migrated to sites of particle deposition ($p<0.05$) and this correlated with an enhanced percentage of phagocytic macrophages following recovery by lavage ($p<0.01$). In vitro functional studies demonstrated that mouse PM phagocytic rates were depressed compared to the other 3 species. The results of chemotaxis studies demonstrated that PM in both rat strains migrated well to zymosan and CI activated sera ($p<0.001$) and lavage ($p<0.05$), while the other 3 species respond best to n-formyl peptides. Our data suggest that the rat is the most efficient model for clearing inhaled particles while hamsters and guinea pigs are more effective in clearing inhaled bacteria.

FATE OF INHALED PARTICLES, DETERMINED BY NEUTRON ACTIVATION - A.P. Wehner, Battelle, Pacific Northwest Laboratories, Richland, WA 99352.

Pulmonary deposition, translocation and clearance of inhaled particles are important parameters in determining biological effects and health risks of air pollutants. Neutron activation provides a very sensitive technique to develop this information, which we have used to determine the fate of talc baby powder and fly ash in hamsters, Mount St. Helens volcanic ash in rats, and cigarette smoke in dogs. The rodent data are reviewed here.

Neutron activation produced radionuclides in the irradiated material, of which ^{46}Sc, ^{59}Fe, and ^{60}Co served as tracers. Rodents received single nose-only exposures to the irradiated dusts. Groups of four to six animals were serially sacrificed up to 4 months after exposure. Lungs, other tissues of interest, and excreta were subjected to γ-ray analysis. Detection limits were ~0.1 disintegrations/min, allowing detection of 10^{-11} g quantities of these elements. Analyzing for more than one radionuclide and comparing their ratios in the bulk dust to those in the tissues or excreta indicated whether a radionuclide represented particles in the tissue samples or had leached from the dust particle.

Six to 8% of inhaled talc was initially retained in the alveoli, with a biological half-life of 7 to 10 days. Alveolar clearance was essentially complete 4 months after exposure. No translocation of talc to liver, kidneys, ovaries, or other parts of the body was found. Picogram quantities of ^{60}Co found in the urine probably represented leached ^{60}Co. Two to 3% of inhaled fly ash was initially retained in the respiratory tract. Estimated biological half-lives were 3 and 35 days for airways and alveoli, respectively. After 99 days, the mean lung burden had decreased to ~10% of its initial value; extrapolation suggests near-complete pulmonary clearance at ~200 days after exposure. About 6% of inhaled volcanic ash was initially retained in the deep lung, with a biological half-life of 39 days. Mean lung burdens had decreased to ~20 and 10% of their initial values 90 and 128 days after exposure, respectively.

CONCENTRATION-TIME RELATIONS IN ACUTE INHALATION TOXICITY.
A THEORETICAL STUDY - A.L.M. Rutten, A. Zwart, P.G.J. Reuzel, TNO-CIVO Toxicology and Nutrition Institute, P.O. Box 360, 3700 AJ Zeist, the Netherlands.

Dose-response relations can be written as $Cexp^n.t$ = constant, which can be transformed to $P = b_0 + b_1 \ln Cexp + b_2 \ln t$, (equation 1), with: P = probit response; b_0, b_1, b_2 constants; $b_1/b_2 = n$; Cexp = exposure concentration; t = exposure time.
We considered theoretical models on distribution and working mechanisms of local and systemical acting agents to determine the range of values of n.

Local- (Agents acting on conductive airways and respiratory epithelium).
a. Agents acting on a location (loc) close to the source of exposure (nose, upper airways): C(loc) is almost immediately Cexp, and is independent of the exposure time; b_2 in equation 1 is 0 and $n = \infty$.
b. Agents acting on a location further away from the source of exposure (alveoli etc): $C(loc) = Cexp\ (1-e^{-kt}-e^{-lt}- \ldots)$ with k, l, ... constants. If k is the largest constant than $n = 1$ for $0<t<2k^{-1}$; $n>1$ for $t>2k^{-1}$ and $n = \infty$ for $t = \infty$.

Systemical- (Agents acting on a target organ (t.o)after having been transported by the blood circulation)
a. Exposed agent is acting agent.
$C(t.o) = Cexp\ (1-e^{-kt}-e^{-lt}- \ldots)$. Again if k is the largest constant $n = 1$ for $0<t<2k^{-1}$; $n>1$ for $t>2k^{-1}$ and $n = \infty$ for $t = \infty$.
b. Exposed agent is metabolised to an acting agent or causes enzyme deactivation.
- Metabolism or deactivation follows first order kinetics:
$\frac{dc}{dt} = \frac{Vm\ Cexp}{Km + Cexp}$ with Vm and Km constants. The amount of metabolite or of deactivated enzyme is / $Cexp\ (1-e^{-kt}-e^{-lt}- \ldots) = Cexp\ (t + k^{-1}e^{-kt} + l^{-1}e^{-lt} + \ldots) - Cexp$.
For $0<t<2k^{-1}$ $n = 2^{-1}$ and for $t>2k^{-1}$ $n = 1$, if k is the largest constant.
- Metabolism or deactivation follows zero order kinetics:
$\frac{dc}{dt} = Vm$
The amount of metabolite or of deactivated enzyme is independent of the exposure concentration; $b_1 = 0$ and $n = 0$.

Conclusions
- A value of n between 0 and ∞ is possible for both local and systemical acting agents.
- n is a function of the range of exposure times and will generally increase for the longer exposure periods for exposed agents that are acting agents.
- For exposed agents that are metabolised to acting agent or that cause deactivation of enzyme Habers rule (P = Cexp.t) with n = 1 can be applied for longer exposure periods.

CATEGORY D
INTERPRETATION OF RESULTS USING LABORATORY ANIMALS
PAPERS

INTERPRETATION OF EARLY LESIONS IN THE MOUSE LUNG: FIBROGENESIS AND TUMORIGENESIS H. P. Witschi, Oak Ridge National Laboratory, Biology Division, Oak Ridge TN 37831

In mouse lung, fibrosis (measured as an increase in total collagen) may develop following acute diffuse lung injury caused by such agents as butylated hydroxytoluene (BHT), bleomycin or cyclophosphamide. It is possible to amplify the fibrotic process by exposing BHT-damaged lungs to X-rays. Presumably, X-ray treatment interferes with repair of the alveolar epithelial lining, allowing fibroblasts to grow uninhibited. However, X-rays failed to potentiate fibrosis following exposure to bleomycin or cyclophosphamide. A reevaluation of cell kinetics following diffuse lung injury showed that it was possible to divide agents into two groups. BHT, methylcyclopentadienyl manganese tricarbonyl, $CdCl_2$ or oleic acid produced peak alveolar type II cell proliferation within 14 days. Cytostatic agents such as bleomycin, cyclophosphamide, BCNU or busulfan produced little cell proliferation. Moreover, alveolar type II cells only began to synthesize DNA 1 to 2 weeks following acute lung injury. It is possible that delayed reepithelization is responsible for the development of fibrosis seen after one single administration of these cytostatic agents.

Lung tumors are derived from type II alveolar cells or from Clara cells, depending on mouse strain. Certain carcinogens such as urethan produce cell hyperplasia whereas others such as 3-methylcholanthrene (MCA) fail to do so. Enhancement ("promotion") of lung tumor development also appears to be independent of cell proliferation. Repeated injections of BHT following a single dose of MCA will promote lung tumors even in the absence of all recognisable cell hyperplasia in the alveolar zone. On the other hand, hyperoxia (70% for 4 months) produces alveolar and bronchiolar hyperplasia and an increase in ornithine decarboxylase activity. The development of tumors is practically abolished. In conclusion, knowledge of cell kinetics in the lung may help on occasion to anticipate a certain pathologic response but also can be misleading in predicting long-term responses. (Operated by Martin Marietta Energy Systems, Inc. with U.S. Department of Energy.)

RELEVANCE TO MAN OF EXPERIMENTALY-INDUCED PULMONARY TUMOURS IN RATS AND HAMSTERS - U. Mohr and D.L. Dungworth, Hannover Medical School, Hannover, FRG and University of California, Davis, CA 95616.

Wide variations in incidence and types of pulmonary tumors among various species of animals are well recognized. In comparing human pulmonary tumors and those induced by respiratory tract exposure of rodents to chemicals, the focus has mainly been on differences in topographic distribution and histogenetic classification. Currently, four mechanistic aspects of pulmonary carcinogenesis are being explored which can help explain differences between human and experimentally-induced rodent tumors: 1) dosimetry of inhaled materials responsible for differences among animal species in anatomic sites of maximum local dose to epithelial cells; 2) variations in epithelial cell populations among species, particularly with respect to xenobiotic metabolism; 3) serial morphologic and functional correlations during carcinogenesis *in vivo*; and 4) studies of events leading to transformation of cultured cells *in vitro*. Comparative dosimetry and epithelial cell biology are considered elsewhere in this Symposium. This paper concentrates on information being developed from *in vivo* and *in vitro* carcinogenetic studies in rats or hamsters.

Activation of oncogenes and expression of their protein products are under intensive investigation as fundamental components of the set of carcinogenetic mechanisms. Studies of lung tumors induced in rats and mice by chronic inhalation of tetranitromethane reveal that the dominant transforming gene is a Ki-ras oncogene activated by mutation at the twelfth codon. This was the case irrespective of tumor type and host species. Since the Ki-ras oncogene appears so far to be the most important activated oncogene in human lung tumors, these findings provide preliminary evidence of the fundamental similarity of the rodent and human tumors. More important is to determine whether the sequence of oncogene activation and expression of products during the complex morphogenesis of lung tumors is similar in humans and animals.

Cell culture studies enable direct *in vitro* comparison of human and rodent cells and can provide quantitative data that can be used in scaling from the animal to man. Evidence to date indicates that fetal human broncho-alveologenic cells are less susceptible to toxic effects and DNA damage by the direct-acting carcinogen ethylnitrosourea. Culture conditions for comparing effects of indirect-acting carcinogens are being explored.

A STUDY OF THE PATHOGENESIS OF EMPHYSEMA ASSOCIATED WITH LONG TERM NO_2 EXPOSURE - J.Kleinerman and M.P.C.Ip, Department of Pathology, Case Western Reserve University School of Medicine at Cleveland Metropolitan General Hospital, Cleveland, OH 44109

Current concepts of the pathogenesis of emphysema are based on the relative balance between elastolytic (E) and antielastolytic (AE) factors. In order to study the dynamic interactions during lung injury we have determined the quantitative change in (E) and (AE) forces in the alveolar lavage fluid of Syrian male hamsters following long term NO_2 continuous and intermittent exposure to NO_2 in concentrations of 30 ppm. The α_1 antielastase (α_1AE) and α_2 macroglobulin (α_2M) concentrations in alveolar fluid and in plasma and the neutrophil (N) and macrophage (M) concentrations in the alveolar fluid (AF) were determined during a continuous 12 month period NO_2 exposure. Studies were done at intervals of 1,3,7,10,14,21 and 30 days and at 3,6,9 and 12 month Plasma (P) and (AF) α_1AE and α_2M were determined by radio immunoassay (RIA). Total (N) elastase (EL) concentration was derived from the (EL) content of an aliquot of purified neutrophils and the total (N) content of the alveolar fluid at each sampling interval. The alveolar (α_1AE) and α_2M concentrations increased markedly within the first 24 hr of NO_2 exposure, as compared to controls (C) thereafter it decreased progressively with continuing exposure, until day 30 and thereafter, when it returned to control levels. The specific activity of the [α_1AE] is markedly depressed after the onset of NO_2, but increases to control values at 30 days. The (P) levels of α_1AE and α_2M during NO_2 show no significant changes from (C). The (N) and (M) content of the AF increases markedly within the first 14 days of NO_2. The (N) concentration returns to near control levels within 30 days, whereas alveolar (M)s decrease more gradually and remain at levels greater than control even after 12 months. Stoichiometric comparisons of the alveolar α_1AE and (N) elastase concentrations indicate an excess of antielastases at all times during the NO_2 exposure. This circumstance would minimize the destruction of alveolar elastin and limit the extent of the emphysema. This supports the pathologic and morphometric findings of minimal emphysema found in the lungs of the NO_2 exposed groups.

Similar studies were performed in hamsters exposed to a series of three intermittent exposures to NO_2 (30 ppm) for 7 days followed by removal from NO_2 for 14 days. The Stoichiometric comparisons of alveolar (AP and (N) elastases again indicate no excess of elastolytic activity at any time during or after the schedule of exposures.

These studies indicate the need to increase the elastolytic stimulus in the lung while simultaneously diminishing the antielastolytic activity in the design of an improved experimental model of human emphysema.

COMPARISONS OF RESPIRATORY FUNCTION RESPONSES OF LABORATORY ANIMALS AND MAN - J.L. Mauderly, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87185.

The respiratory function of man is measured in clinical, occupational and epidemiological settings to detect and characterize functional abnormalities and to follow the progress of lung disease. The pulmonary health of man is often described solely on the basis of measured function. Methods have been adapted so that function parameters measured in man can be measured in animals, including multiple indices of breathing patterns, lung volumes, parenchymal and airway mechanics, intrapulmonary gas distribution, alveolar-capillary gas exchange, pulmonary perfusion and blood gases. Specific techniques used for man and animals often differ, due primarily to the need for animal restraint and the inability to obtain voluntary, controlled respiratory movements from animals. However, tests of animals rarely need to be invasive beyond anesthesia, many can be performed without anesthesia, and all can be performed repeatedly. Because of differences in measurement technique and because the lungs of man and animals differ structurally, it is important to determine if measured functional responses to airway irritants and lung injury are similar in animals and man. Data on functional responses of man and animals to inhaled irritants, bronchoactive drugs, and pulmonary diseases (inflammation, emphysema and fibrosis) will be reviewed. Data from man and animals with radiation-induced pneumonitis and fibrosis and dust-induced pneumoconiosis will also be compared. This information indicates that similar lung injuries result in qualitatively similar alterations of respiratory function in man and laboratory animals, and that there are also many quantitative similarities. Respiratory function assays are a useful tool in inhalation toxicology as a response endpoint, to detect and characterize lung disease, to study morphologic-physiologic correlates and to place the results of animal studies in a context that can be extrapolated to man.. (Research supported by the Office of Health and Environmental Research, U.S. Department of Energy under Contract No. DE-AC04-76EV01013.)

NASAL TUMOURS IN RATS AFTER SHORT-TERM EXPOSURE TO CYTOTOXIC CONCENTRATIONS OF FORMALDEHYDE VAPOUR - V.J. Feron, L.M. Appelman, R.A. Woutersen and J.P. Bruyntjes, TNO-CIVO Toxicology and Nutrition Institute, P.O. Box 360, 3700 AJ Zeist, The Netherlands

Formaldehyde vapour has been shown to be carcinogenic to rats and probably also to mice (Swenberg, J.A. et al., Cancer Res., 40, 1980, 3398; Albert, R.A. et al., J. Natl. Cancer Inst., 68, 1982, 597; Kerns, W.D. et al., Cancer Res., 43, 1983, 4382).
To study the chronic effects on the nose of exposure to clearly cytotoxic concentrations of formaldehyde during relatively short treatment periods, a long-term inhalation study was carried out in male rats using exposure levels of 0, 10, and 20 ppm and exposure periods of 1, 2 or 3 months (6 h/day, 5 days/week) followed by observation (non-exposure) periods of at most 29, 28 or 27 months, respectively. A total of 450 rats was used. Preliminary results indicate the occurrence of compound-related inflammatory, hyperplastic and metaplastic changes of the nasal respiratory epithelium even after a non-exposure period of 29 months. Grossly visible nasal tumours were not found. However, microscopic examinations carried out so far indicate the presence of a limited number of nasal tumours in rats of the high-concentration group.

A RAT MODEL OF PHYSIOLOGIC ADAPTATION TO OZONE (O_3) - D.L. Costa,* J.S. Tepper, M.J. Wiester,* J.R. Lehmann, M.F. Weber, S. Fitzgerald, M. Stevens, M.E. King. *Health Effects Research Laboratory, U.S.E.P.A., RTP, NC 2771; Northrop Services Inc., RTP, NC 27709.

Repeated daily exposure of humans to O_3 evokes lung dysfunction which subsides or "adapts" by the 3rd or 4th day of challenge. To investigate morphological and biochemical alterations which may be related to these functional changes, a rat model of adaptation was developed. Male, F-344 rats were exposed to 0.0, 0.35, 0.5, or 1.0 ppm O_3 for 2.25 hrs each of 5 consecutive days during which 8% CO_2 was superimposed for alternate 15 min periods to stimulate ventilation as does exercise in the human protocols. Observed concentration-dependent alterations in tidal breathing parameters were largely adapted by day 3 in the 0.35 and 0.5 ppm group, but were minimally attenuated at 1.0 ppm. In a second study, groups of rats were exposed to 0.0 or 0.5 ppm O_3 as above, but were not repeatedly monitored for functional changes. Instead, separate cohort groups were evaluated each day. Preliminary functional evaluations indicated less adaptation than previously observed, but this may reflect the use of cohort groups. Immediately postexposure the animals were killed and lungs were excised and weighed. The right lobe set was lavaged with sterile saline while the left lobe was fixed with 2% glutaraldehyde at 25 cm H_2O. Lavagate determinations included: albumin and glucose as permeability markers, cell counts and differentials, and α-1-antiproteinase (α1-Pi) function. Freely permeable urea was used to calculate the lung lining layer volume. Lavagable cell populations did not change, but macrophage phagocytosis was suppressed without evidence of "adaptability". Permeability, α1-Pi function and lung structure analyses are in progress. These data will be used to correlate physiologic "adaptation" to O_3 with underlying organic processes. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

CENTRIACINAR AIRWAY REMODELING IN RATS CHRONICALLY EXPOSED TO OZONE - D.M. Hyde, B.C. Barr, C.G. Plopper and D.L. Dungworth, California Primate Research Center, University of California, Davis, CA 95616.

The morphologic changes in the centriacinar region of lungs from 14 rats exposed to either filtered air (8) or 0.95 ppm ozone (6) eight hours daily for 90 days were analyzed using morphometry. Rats were killed with an overdose of sodium pentobarbital administered I.P., the trachea cannulated and thoracic viscera and lungs removed from the chest. Lungs were fixed via intratracheal instillation of a paraformaldehyde/glutaraldehyde cacodylate buffered fixative at 30 cm water pressure. Lung volumes were determined by weight displacement and the left lung lobe was sectioned transversely into 12 slabs. One random block was selected from each slab, and 12 blocks per rat were embedded in paraffin and studied by light microscopy. With these sections we estimated the volume of proximal bronchiole, the segment proximal to the terminal bronchiole, terminal bronchiole, respiratory bronchiole and alveolar duct and sac within the lung. Bronchioles dissected from pre-selected regions of the right middle lobe were studied by transmission electron microscopy. Dissected terminal airways were sectioned in a longitudinal plane through their mid-lumen. From these dissected airways, four subregions of the centriacinus were then examined: 1) terminal bronchiole, 2) respiratory bronchiole, 3) centriacinar alveolar duct wall and 4) centriacinar alveolar septa. The results of this study showed that following chronic ozone exposure there was a 13-21% decrease in luminal diameter and a 32% decrease in luminal volume of terminal bronchioles. The volume of proximal bronchiole was unchanged in volume. The most notable change was a 3.4-fold increase in respiratory bronchiole volume. While the walls of terminal and respiratory bronchioles were significantly thicker, the centriacinar alveolar duct wall was 90% thicker and showed a marked increase in cuboidal epithelial cells. The results indicate that respiratory bronchiole is formed from centriacinar alveolar duct following chronic exposure to ozone, and that the narrowing of terminal bronchioles at 30 cm of water pressure fixation may reflect decreased compliance of the bronchiolar wall.

Supported by NIEHS Grant P01 ES00628.

ROLE OF VITAMIN E IN THE DIFFERING RESPONSE TO NO_2 INJURY IN THE RAT AND THE HAMSTER - K. Miller, D. Baylcy and D.G. Walters, British Industrial Biological Research Association, Carshalton, Surrey, SM5 4DS, U.K.

Pulmonary surfactant may exercise a dual role both in maintaining alveolar stability and in protecting the respiratory membrane against the deleterious effects of lipid peroxidation and other toxic injury. We have shown previously that hamster and rat differ in their response to NO_2 exposure and that vitamin E is present in surfactant obtained from lavage fluid or whole lung homogenate in both species. Vitamin E levels were found to be appreciably higher in the rat than hamster and could thus play a role in protecting against NO_2 toxicity in the rat.

We have now investigated whether the two species differ in their ability to utilise vitamin E on animals maintained on diets supplemented with vitamin E (375mg/kg diet) for a 4 week acclimatization period prior to NO_2 exposure.

Administration of vitamin E supplemented diet increased both plasma concentration and the level of vitamin E present in whole lung in the rat. This effect differed from that obtained in the hamster where administration of vitamin E increased plasma concentration three-fold but had no effect on the level of vitamin E in whole lung surfactant. This suggests that in addition to higher levels of vitamin E in the plasma the rat may differ from the hamster in its ability to utilize vitamin E pools and transport them to other organs such as the lung. This might provide some explanation for the greater susceptibility of the hamster to the NO_2 injury.

DOSIMETRIC CONSIDERATIONS FOR EXTRAPOLATING RESULTS OF RAT INHALATION STUDIES TO HUMANS: CADMIUM CARCINOGENICITY STUDIES AS AN EXAMPLE - G. Oberdörster, University of Rochester, Dept. of Biophysics, Rochester, NY 14642.

Interpretation of results from rat inhalation studies and extrapolation of those results to humans requires knowledge in both rats and humans about regional deposition efficiencies in the respiratory tract, pulmonary retention half times of the chemical in question, the metabolism of the compound in the respiratory system and the sites of action within the respiratory tract. Inhaled cadmium compounds have been shown to induce lung tumors in both rats and man, however, it appears that the tumorigenic effect is much greater in rats than in man. Tumors induced in rats by inhalation of cadmium seemed to be mostly of peripheral origin, whereas in humans inhaled cadmium induced mostly tumors of bronchogenic origin. Model deposition calculations were performed assuming an eight hr/day exposure to cadmium aerosols of different particle sizes, heterodispersity and concentrations. For a submicrometer size cadmium aerosol - the particle size used in rat studies - the deposited dose per unit surface area in the transitional region of the lung when inhaled via the nose is about 2 times higher in the rat than in man, whereas the surface area dose in the conducting airways of both species is about the same. Inhalation of a larger sized cadmium aerosol - often encountered under workplace conditions, e.g., exposure to CdO dust with a median particle size of 3µm - leads to a doubling of the surface area dose in the upper conducting airways of man. Mouth breathing increases the dose per unit surface area in generations 2-6 of the human lung significantly by an additional factor of five to six. Since pulmonary retention of Cd differs significantly between rats (T 1/2 = 80 days) and primates (T 1/2 = 770 days) this has also to be considered for long-term accumulation of Cd in the lung. Respective model calculations show that the accumulated dose of Cd per g of lung tissue is greater in man by a factor of two to five. However, the dose rate per g of lung tissue during cadmium aerosol exposure is more than fourfold greater in the rat. These results demonstrate that differences in the sites of tumor induction between rats and humans by inhaled cadmium may be due to differences in the surface area dose and the dose rate. Such dosimetric differences should be considered when designing inhalation experiments in rats and extrapolating the results to humans.

CAN ANIMAL DATA RELATE TO HUMAN BRONCOGENIC EFFECTS?

J.N. Pritchard and A. Black

Environmental & Medical Sciences Division, A.E.R.E. Harwell, Oxon, England

Despite the strong epidemiological evidence linking cigarette smoking to bronchogenic carcinoma, it has proved very difficult to reproduce this effect in laboratory animals. This suggests that the response of laboratory animals may be a poor indicator of risk to the human bronchial region. As there is evidence to suggest that the site of deposition is related to the site of origin of tumours, this probably reflects a different pattern of deposition in the animals. This paper will discuss the factors that may be responsible for differences in deposition between humans and animals. In particular, the effects of deposition in the upper airways and residence time in the lung will be considered, using the deposition of cigarette smoke as an example. The behaviour of volatile and hygroscopic materials will be discussed in this context.

CATEGORY D
INTERPRETATION OF RESULTS USING LABORATORY ANIMALS
POSTERS

The NTP/NIEHS STUDIES ON METHYL ISOCYANATE - J.R. Bucher, E.E. McConnell, B.A. Schwetz, M.D. Shelby, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Prior to the December, 1984, release of methyl isocyanate (MIC) in Bhopal, India, few toxicologic studies had been performed on MIC, and the human health consequences for the exposed population were unknown. Because of this, the U.S. National Toxicology Program, and National Institute of Environmental Health Sciences performed studies with rats and mice to characterize respiratory injury and to assess the potential for systemic toxicity through histopathologic studies and assessments of immune, reproductive and genetic effects. MIC inhalation in single 2-hour exposures, or in repeated, 6-hour exposures over 4 days, resulted in immediate and delayed deaths, both attributed to respiratory distress. Immediate deaths were associated with sloughing of the epithelium of the respiratory tract to the level of the bronchioles. In survivors, the nasal mucosae rapidly healed, but small airways remained occluded with mucus, fibrin, desquamated cells and fibropepithelial scars. Function studies demonstrated severe-persistent obstructive lung disease with secondary complications of pulmonary hypertension and cardiac arrhythmias. However, little evidence of direct systemic effects was found. MIC-exposed mice showed transient decreases in bone marrow cellularity and hematopoietic progenitor cells, but no functional deficits in host resistance to bacterial, viral or malarial challange were seen. Exposure during late pregnancy resulted in increased fetal and neonatal deaths, but no evidence of reduced fertility or teratogenic effects was found. Results of in vitro and in vivo genetic toxicity assays were mixed and the conclusion that MIC is weakly genotoxic was based primarily on increases in chromosomal aberrations and sister chromatid exchanges both in cultured mammalian cells and in bone marrow of exposed mice. Despite the fact that the exposures in these animal studies were to pure MIC, whereas the Bhopal exposures presumably were to MIC and various reaction products, these experimental findings are quite consistent with published reports of the medical conditions of survivors of the Bhopal disaster.

EARLY RESPONSE OF THE SURFACTANT AND ANTIOXIDANT SYSTEMS IN RAT LUNGS AFTER CADMIUM CHLORIDE INHALATION.

J Boudreau, R. Vincent, D. Nadeau*, B. Trottier, M. Fournier, K. Krzystyniak and G. Chevalier, Université du Québec à Montréal, QC, Canada, H3C 3P8 and *Université de Sherbrooke, QC, Canada, J1K 2R1.

We are developing an animal model for assessing the early effects of inhaled pollutants on the lungs in terms of sensitive and specific biochemical parameters. Male Long-Evans rats were exposed to a $CdCl_2$ aerosol ($5mg/m^3$) for 1 hr and were sacrificed at 1, 4, 8, and 16 days after treatment. The toxicity was assessed by studying the responses of the tissue antioxidant defence and the pulmonary surfactant (SF) systems. Pulmonary edema (wet/dry wt) was observed only on day 1. The total activities of superoxide dismustase (SOD), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD) in the lung homogenates (HOM) increased (125%-250%; $p<0.05$; n=8) throughout the study. Significant increases in the specific activities (U/mg protein or dry wt) of G6PD were observed on days 1 and 4, suggesting an oxidative stress. The general increases in GR and SOD total activities seemed associated with the infiltration by inflammatory cells, as indicated by the increased levels of acid phosphatase and B-N-acetylglucosaminidase. The response of the SF system was monitored by assaying the alkaline phosphatase activity (AKP) and the phospholipid (PL) content in HOM and cell-free bronchoalveolar lavages (BAL). The AKP activity in the HOM decreased (30%) on day 1, while no activity could be detected in the BAL, indicating an inhibition of AKP by Cd. The recovery of PL in the BAL decreased by 44% on day 1 although the lung PL content increased by 61%, suggesting an alteration in SF secretion. On day 4, the high recovery of PL in the BAL (312%) may reflect a reduced recycling and/or increased secretion of extracellular SF. Also, the important changes in the AKP specific activity (U/mg PL) observed on days 4 (42% decrease), 8 (262% increase), and 16 (38% decrease) were interpreted as an indication of qualitative changes in extracellular SF. Cadmium intoxication appears to alter significantly the pulmonary SF system and to produce an oxidative stress. The AKP activity is particularly sensitive to Cd, and may possibly provide an early and sensitive indicator of SF alterations. (Supported by NSERC and IRSSTQ).

EFFECTS OF A "NUISANCE" DUST INHALATION ON LAVAGABLE LUNG CELLS - O. Creutzenberg, B. Bellmann , M. Ocak and H. Muhle, Fraunhofer-Institut für Toxikologie und Aerosolforschung, Hannover, FRG.

Particles for which no specific toxic effects are known are classified as "nuisance" dusts. A general threshold limit value has been established for an occupational exposure to those aerosols amounting to 6 mg/m^3 in Germany and 5 mg/m^3 in the U.S. respectively.
The aim of the study was to investigate effects of a long-term exposure to nuisance dusts. In addition to lung clearance measurements, the functions of the alveolar leucocytes were studied. Three dusts were tested in parallel. Test materials were titanium dioxide, polyvinyl chloride powder (PVC) and iron powder at aerosol concentrations of 3.2, 8 and 20 mg/m^3. The exposure lasted up to 8 months. The used animals were female Fischer rats. Ten animals of each concentration group were taken for lung lavage. Lavagable lung cells were examined for the following parameters: total cell number, cell viability, phagocytic activity, adherence and chemotaxis tests, peroxidase staining and differential cell counting. The results showed an almost constant number of total cells over all groups except the highest PVC concentration group, the cell number of which increased significantly. The number of polymorphonuclear cells increased from low to higher concentrations. The results indicate more pronounced effects by PVC powder compared to TiO_2 and Fe dust. Previous experiments during exposure of the animals have shown that PVC reduces alveolar lung clearance. The relevance of these effects for the general threshold limit value for dusts will be discussed.

METHYL ISOCYANATE (MIC) TOXICITY IN RATS AND GUINEA PIGS FOLLOWING ACUTE, HIGH CONCENTRATION EXPOSURE - D.E. Dodd, M.R. Fedde, E.H. Fowler, C.M. Troup, L.A. Maginniss, and F.R. Frank, Bushy Run Research Center, Union Carbide Corporation, Export, PA 15632

Early reports from India indicated that humans were dying within minutes to a few hours from exposure to MIC. To examine the probable causes of these rapid mortalities, studies involving rats and guinea pigs focused primarily on the consequences of acute pulmonary damage. All MIC inhalation exposures were acute, of short duration (mainly 15 min.), and high in concentration (ranging from 25 to 3500 ppm). The MIC vapor exposures were statically-generated in a double chamber design. Guinea pigs were more susceptible than rats to exposure-related early mortality. A greater than one order of magnitude difference was observed between an MIC concentration that caused no early mortality in rats (3506 ppm) and an MIC concentration that caused partial (6%) early mortality in guinea pigs (225 ppm) for exposures of 10 to 15 min. duration. Although rat and guinea pig packed erythrocytes exposed in vitro to 100, 500, 1000, or 2000 ppm of MIC vapor had a concentration-related inhibition of cholinesterase activity, in vivo exposure to 1000 ppm of MIC did not result in inhibition of erythrocyte cholinesterase activity. Also, no direct effects of MIC on hemoglobin function were observed in guinea pigs exposed to 700 ppm for 15 min. However, blood O_2 affinity was reduced due to severe metabolic acid-base disturbances (lactic acidosis). Guinea pigs exposed to MIC at concentrations of 240 to 628 ppm had a marked reduction in PaO_2 and pHa and an elevated tracheal pressure during artificial ventilation. The low PaO_2 was only slightly elevated when the animals were ventilated with 100% O_2. Thus, MIC inhalation caused severe pulmonary blood shunting and ventilation/perfusion imbalance. This, in turn, led to hypoxemia, metabolic acidosis, and tissue hypoxia, which could produce death. The pulmonary gas exchange deficit presumably resulted from sloughing of large sheets of conducting airway epithelium and mucus production resulting in plugging of major airways. Exposure-related degenerative changes were also seen in the alveolar epithelium and the endothelium in both rats and guinea pigs. However, the guinea pig was considerably more sensitive to MIC vapor.

EICOSANOID PRODUCTION BY RABBIT ALVEOLAR MACROPHAGES AFTER IN VITRO AND IN VIVO OZONE EXPOSURE - K.E. Driscoll and R.B. Schlesinger, Institute of Environmental Medicine, New York University Medical Center, NY, NY 10016.

This study was designed to examine the effect of ozone on alveolar macrophage (AM) eicosanoid biosynthesis and to compare the responses observed using in vitro and in vivo exposure techniques. AM were obtained from New Zealand White rabbits by bronchoalveolar lavage, established in monolayer culture, and exposed for 2 hr to atmospheres containing 0.0, 0.1, 0.3, or 1.2 ppm ozone. The AM were maintained in culture for 6 hr post exposure, the conditioned media harvested, and analyzed for the presence of PGE2, PGF2 α, PGF1α thromboxane, LTB4 and LTC4 using radioimmunoassay. For the in vivo exposure study, groups of 5 rabbits were given a single 2 hr exposure to air, 0.1, or 1.2 ppm ozone and sacrificed immediately or 24 hr post exposure. AM lavaged from the exposed rabbits were cultured for 6 hr and the conditioned media analyzed for eicosanoids. In vitro exposure to ozone resulted in a significant increase in PGE2 and, to a lesser extent, PGF2α production at both the 0.3 and 1.2 ppm levels; no significant changes in PGF1α production were observed for any of the ozone concentrations. LTB4, LTC4 and thromboxane could not be detected after ozone exposure. Regression analysis of the in vitro exposure PGE2 and PGF2α data demonstrated a significant linear concentration-response relationship with the slope of the PGE2 regression being significantly greater than that of PGF2α. Preincubation of AM with 10 µg/ml indomethacin inhibited prostaglandin synthesis after in vitro ozone exposure. In vivo exposure to 1.2 ppm resulted in significantly increased quantities of PGE2 and PGF2α released at both the immediate and 24 hr sacrifice points. After in vivo exposure to 0.1 ppm, no change in prostaglandin production was observed. These results demonstrate that both in vitro and in vivo ozone exposure stimulates eicosanoid production by rabbit AM. The responses observed using the two exposure techniques were qualitatively similar, with quantitative differences likely due to the higher effective ozone dose which would be expected using the in vitro system. Increased production of prostanoids (particularly PGE2) may affect respiratory defense mechanisms. PGE2 is known to down-regulate the functional activities of macrophages and other immune effector cells. Increased levels of this prostanoid in the alveolar region represents a potential mechanism for the impaired pulmonary defenses which have been observed after ozone exposure.

MORPHOGENESIS OF THE LUNG CANCER INDUCED BY DIESEL EMISSION - S.Kitamura,Y.Takaki,N.Kuwabara and Y.Fukuda,Juntendo University School of Medicine,Hongo,Bunkyo-ku,Tokyo,Japan.

In order to observe the carcinogenesis of diesel emission, long-term inhalation studies were carried out on SPF F-344 rats (4 week of age) using a heavy-duty (11 liter) and light-duty (1.8 liter) diesel engin.Each exhaust gas was diluted to five levels concentration. A group of rats consisted of 120 males and 120 females for each level of exhaust gas concentration. Rats were exposed 16 hours/day, 6 days /week for up to 30 months to exhaust gas. The experimental rats were kept microbiologically so clean and the rats showed no evidence of bacterial infection of respiratory organ.

Histolgicaly,the diesel particles were deposited in the interstitial tissue and phgocytosed by alveolar macrophages along with alveolar septum.These changes were dependent on the duration of exposure and the exhaust gas concentration. Hyperplasia of the type II alveolar epithelial cells and bronchiolar epithelium was increased with varying degrees of anthracosis.This hyperplasia was a focal and scattered lesion until 12 months inhalation.However,over 18 months inhalation,those hyperplastic lesions extened to the adjacent alveolar spaces and fused with the other focal lesions turning into a diffuse pattern. In such diffuse hyperplastic lesion,there was papillary and solid epithelial proliferation with slight cellular atypia without nodular formation or compression of the adjacent tissue. Some of these findings were difficult to differentiate from adenoma and occasionally even the carcinoma.Over 24 months,squamous metaplasia was often observed in subpleural hyperplastic lesion with focal interstitial fibrosis.

The incidence of primary lung tumors including adenoma, carcinoma and other tumors was dependent on heavy-duty diesel engin.However, the incidence was independent on light-duty diesel engin. The majority of these tumors were squamous cell carcinoma and adenosquamous carcinoma. Pure adenocarcinoma was few in number.

It can be concluded that morphogenesis of the lung cancer which is induced by diesel emission is related to hyperplasia and squamous metaplasia of bronchiolar-alveolar epithelial cells as precursorous changes.

THE EFFECT OF DUST INHALATION ON BACTERIAL CLEARANCE FROM THE LUNG - M.I. Gilmour, F.G.R. Taylor and C.M. Wathes
School of Veterinary Science, University of Bristol, Langford House, Langford, Bristol, BS18 7DU, England.

Exposure to airborne dust may compromise the respiratory defences of farm livestock by blocking non-specific and specific clearance mechanisms, thus predisposing to opportunistic and primary pathogens. To test this hypothesis we are examining the clearance of a bacterial infection, and the immune response to that infection, during exposure to airborne dust.

We have developed a model of respiratory infection in the mouse using Pasteurella haemolytica. Mice are subjected to various concentrations of an inorganic dust, titanium dioxide, and infected with P. haemolytica by aerosol. The bacterial clearance, specific serum antibody and lymphocyte sensitivity to Pasteurella antigen are monitored during two weeks post-infection.

Prior exposure to airborne dust impaired bacterial clearance. The effects on the immune response subsequent to infection will be discussed. We conclude that dust particles of a respirable size adversely affect clearance mechanisms, and that the effect is dose-related.

COMPARISON OF RESPIRATORY FUNCTIONS IN FISCHER 344 AND WISTAR RATS - U. Heinrich and A. Wilhelm, Fraunhofer-Institut für Toxikologie und Aerosolforschung, Hannover, FRG

Various strains of Fischer 344 and Wistar rats have been used in basic research and toxicological tests of all designs and it is well known that these two strains differ in some biological data, e.g. growth rate, clinical chemistry, hematology, spontaneous tumors a.o.
In inhalation toxicology, lung function measurements are often used to assess toxic effects of inhaled material on pulmonary mechanics and diffusion. But, so far, there are no investigations comparing normal lung function of Fisher 344 and Wistar rats.
Therefore, we started to measure various lung functions (mechanics and diffusion) in groups of female Wistar [Han: WIST] and Fischer [CDF (F-344) CrlBR] rats using the whole-body plethysmograph and halothane anesthetised animals breathing spontaneously through a transorally inserted tracheal cannula. Spontaneous breathing-related lung mechanics, maximum flow-volume- and quasistatic lung pressure-volume relationships, subdivisions of lung volume and CO diffusing capacity were measured. The animals were investigated at the age of approx. 4, 13 and 18 months.
The most striking difference between these two strains of rats was the significantly higher lung volume of the 18 weeks old Wistar rats compared to the 17 weeks old Fischer rats, both having a mean body weight of 220 g. This difference in the lung volume was even more pronounced approx. 1 year later, although, the mean body weight of both strains of rats had increased by 50%. But, only the size of the lung and not the mechanical property appeared to be different because lung compliance and resistance normalized to functional residual capacity was very similar in Fischer and Wistar rats.

ELECTRON MICROSCOPIC STUDIES ON RAT LUNGS AFTER PROLONGED EXPOSURE TO DIESEL ENGINE EXHAUST, A.KATO, S.ISHIWATA, (HERP), Japan Automobile Research Institute, Inc., Yatabe, Tsukuba-gun, Ibaraki, Japan.

Inhalation experiments on diesel engine exhaust were performed for 30 months using F344 rats. The results of electron microscopic observations (TEM.SEM) on the respiratory organs are reported here.

In the particle-containing groups, the main changes of the airway surface (SEM) during the 30 months of inhalation were irregularity, shortening or loss of cilia of the ciliated epithelia, and irregularly arranged protrusion or hypertrophic foci of the non-ciliated epithelia. The changes of the cilia were prominent in the trachea or main bronchi, however, the changes of the non-ciliated cells were perdominantly focused on the Clara cells of more the distal regions of the airways.

In the alveoli (TEM), acceleration of phagocytotic activity at the surface of type I epithelia and uptake of particles into the attenuated cytoplasm were noticed. Proliferation of hypertrophic type II epithelia was noted with increased microvilli and swollen or fused lamella inclusion bodies. Slight increase of collagen fibers, edematous swelling and carbon laden macrophages in the interstitium of the alveolar walls were seen occasionally. In the alveolar lumen, macrophages and laminated materials discharged from the type II epithelia were observed. These changes in the airways and alveoli were more prominent at the high dose groups of both the LD and HD series. These lesions also increased slightly with the lengths of inhalation time. On the other hand, the particle influence experiment gave rise to a similar extent of lesions both in the particle-containing and the corresponding particle-free groups. Thus, the lesions of the airway epithelia seemed to be caused mainly by the effects of gaseous components.

Finally, electron microscopic morphometry showed a dose-dependent increase of the arithmetic mean thickness of the air-blood barrier only in the groups in the LD series after inhalation for 6 and 12 months. However, after 30 months of inhalation, some groups showed marked variations in alveolar wall thickness, due to infiltration of leukemic cells into the interstitium and slight fibrosis. Therefore, it was difficult to differentiate the changes caused by diesel emissions.

NASAL TUMOURS IN RATS AFTER SEVERE INJURY TO THE NASAL MUCOSA AND EXPOSURE TO FORMALDEHYDE VAPOUR - V.J. Feron, H.R. Immel, L.M. Appelman, R.A. Woutersen and A. Zwart, TNO-CIVO Toxicology and Nutrition Institute P.O. Box 360, 3700 AJ Zeist, The Netherlands

Prolonged exposure to formaldehyde vapour may induce nasal tumours in rats (Swenberg, J.A. et al., Cancer Res., 40, 1980, 3398; Albert, R.A. et al., J. Natl. Cancer Inst., 68, 1982, 597) and probably also in mice (Kerns, W.D. et al., Cancer Res., 43, 1983, 4382). To study the significance of damage inflicted upon the nasal mucosa for the induction of nasal tumours by formaldehyde, a long-term inhalation study was carried out in which male rats with a severely damaged (by electrocoagulation) or undamaged nasal mucosa were exposed to 0, 0.1, 1.0 or 10 ppm formaldehyde vapour for 6 h/day, 5 days/week during a period of 30 months, or a period of 3 months followed by a non-exposure period of 27 months. The total number of rats was 880. Preliminary results suggest that chronic exposure of rats to 1 or 10 ppm formaldehyde leads to a higher incidence of nasal carcinomas in rats with a damaged nasal mucosa than in rats with an intact nasal mucosa.

USE OF BRONCHOALVEOLAR LAVAGE TO ASSESS THE TOXICITY OF AIRBORNE MATERIALS - R. F. Henderson and R. O. McClellan, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87185

The epithelial lining fluid (ELF) of the respiratory tract is the first substance encountered by inhaled materials. Analysis of this fluid and the cells associated with it can be used to detect the initial response of the respiratory tract to the inhaled material. ELF can be sampled by bronchoalveolar lavage (BAL). This technique has proven useful in three areas of toxicologic research: 1) BAL can be used to detect an acute inflammatory response in the bronchoalveolar region. This approach has been used to rank the pulmonary toxicity of a series of compounds (for example, a series of nickel salts) and to determine the amount of a material in the lung required to elicit an inflammatory response. 2) BAL can be used to follow the progress of a lung injury sequentially to determine if the initial inflammatory response leads to normal repair or to chronic inflammation and development of irreversible lung pathology. It has been used in this manner to determine if the acute response of the lung to inhaled particles is predictive of the long-term effect of the particles retained in the lung. 3) BAL can be used to elucidate the pathogenesis of various pulmonary diseases. Analysis of ELF for parameters such as mediators of inflammation, growth factors, protease/antiprotease balances has been an aid in determining the processes by which lung pathology develops. Advantages of BAL are that it is quantitative, allows detection of early changes in the respiratory tract, it can be done serially, and it is integrative in that it detects inflammation at any point in the luminal portion of the bronchoalveolar region. Disadvantages are that it is an invasive procedure and results are not site specific. BAL represents a valuable additional tool by which one can assess the response of the respiratory tract to inhaled toxins and is complementary to established methods such as histopathology, radiology, and pulmonary function assessment. [Research performed under U.S. Department of Energy Contract No. DE-AC04-76EV01013.]

THE LARYNX AS A POTENTIAL TARGET ORGAN IN AEROSOL INHALATION STUDIES ON RATS - D.R. Klonne, R.H. Garman, W.M. Snellings, D.E. Dodd, and B. Ballantyne, Bushy Run Research Center, Export, PA 15632.

The larynx has been observed to be a target organ for the particulate fraction, but not for the vapor phase, of cigarette smoke (Coggins et al., Toxicol. 16:83, 1980). In conducting 2-, 4-, and 13-week liquid aerosol inhalation studies (6 hr/day; MMAD ≤ 4.5 μ) on an aqueous silane solution (gamma-methacryloxypropyltrimethoxysilane), laryngeal granulomas were produced in F-344 rats after as few as 9 exposures to a mean aerosol concentration of 50 mg/m^3. Rats exposed to vapors of the same material for 9 exposures at mean concentrations up to 426 mg/m^3 exhibited no laryngeal toxicity. In neither aerosol nor vapor studies was there an indication of lung toxicity. In the 4-wk aerosol study, scanning electron microscopy (EM)/energy dispersive X-ray analysis evaluations confirmed the presence of siliceous spheres of approximately 2 μ in diameter in the submucosa of the larynx. The laryngeal granulomas appeared as a polypoid protrusion of the overlying mucosa into the lumen of the airway. The siliceous spheres were often surrounded by large numbers of epithelioid histiocytes with smaller numbers of giant cells also present. Transmission EM analysis indicated that the siliceous spheres were present in the cytoplasm of squamous mucosal cells, within histiocytes and fibroblasts in the submucosa, and extracellularly in the submucosa. The size of the granuloma remained nearly constant in rats allowed a 13-wk non-exposure recovery following 13 weeks of exposure at concentrations ranging from 50 to 244 mg/m^3.

Guidelines (TSCA, FIFRA, OECD) for subchronic inhalation aerosol studies do not specifically require that the larynx be histologically evaluated. Furthermore, the site of the lesion appears to be quite specific, occurring posterior to the epiglottis in the ventral portion of the larynx at the level of the vocal cords and immediately adjacent to the ventral laryngeal diverticulum. Because of the serial sections required to achieve the specific site, a single random section through the larynx would often miss a lesion unless it were quite large. The results of these studies raise the question of whether the larynx is a potential target organ for aerosols of silane compounds, or for many aerosol test compounds, because of an unusual cellular sensitivity or an increased deposition and/or decreased clearance of particles in this area in the rat.

SUBCHRONIC INHALATION STUDY OF PIGMENTED POLYMER IN RATS - H. Muhle[1], U. Mohr[1], S. Takenaka[1], W. Koch[1], R. Fuhst[1], R. Kilpper[2], and R. Mermelstein[2]

1. Fraunhofer-Institut fur Toxikologie and Aerosolforschung, Hannover 61, F.R.G.,

2. Joseph C. Wilson Center for Technology, Xerox Corporation, Rochester, New York, 14644, U.S.A.

As part of a long-term investigation, a subchronic inhalation study of a pigmented polymer fraction was conducted by exposure of groups of SPF F-344 rats for six hours/day, five days/week for 13 weeks. The test material is a special 9600-type xerographic toner enriched about 10-fold in respirable size particles such that it is about 35% respirable according to ACGIH criteria. The nominal aerosol exposure concentrations were 0, 1.0, 4.0, 16.0 and 64.0 mg/m^3.

The animals tolerated the exposure conditions well; no unscheduled deaths occurred. Body weight, clinical chemistry values, food consumption and organ weights were normal with the exceptions noted for the highest exposure group. Constant body weight and clinical chemistry values, slight decrease in food consumption and about 50% increase in lung weight were observed in the highest exposure group.

Histopathological examination of the lungs indicated a dose related increase of particle accumulation and macrophage recruitment. A very slight degree of septal thickening of the alveolar structure was noted in the highest exposure groups.

LONG-TERM INHALATION STUDY WITH HAMSTERS AND MICE USING VARIOUS CADMIUM COMPOUNDS - U. Heinrich, R. Fuhst, H. König, L. Peters and S. Takenaka, Fraunhofer-Institut für Toxikologie und Aerosolforschung, Hannover, FRG

Inhalation of 12.5, 25 and 50 µg cadmium/m^3 in $CdCl_2$ for about 150 hrs/week for 18 months induced lung carcinomas in rats (Takenaka et al. 1983). Therefore, the carcinogenic effect of $CdCl_2$ and three other Cd-compounds ($CdSO_4$, CdO, CdS) and the susceptibility of other species (hamsters, mice) merited investigation. In this experiment, male and female Syrian golden hamsters and female mice (NMRI) were exposed to aerosols of four different Cd-compounds on 5 days/week, for 19 hrs/day or 8 hrs/day for 12 - 16 months. After termination of the exposure, the animals were kept in clean air for another 6 - 12 months. The Cd-exposure was terminated prematurely if there was a substantial loss of body weight or increased mortality. The preliminary results of this experiment are:
After exposure to the different Cd-compounds for approx. one year followed by a clean air period of almost another year at the most, no neoplastic alterations in the respiratory tract of the hamsters have been observed up to now. The spontaneous lung-tumor rate in the lungs of the control mice was about 32 % after an experimental time of two years. No additional tumor-inducing or inhibiting effect could be observed after Cd-exposure.
Additionally, the Cd-content of the lung, liver and kidney was determined and correlated to the experimental time.

ENDOGENOUS LIPID PNEUMONIA INDUCED BY METHYLNAPHTHALENE, A COMBUSTION GAS OF KEROSINE, IN $C_6B_3F_1$ MICE

Y. Konishi*, Y. Emi*, T. Taki**, Y. Murata* and Y. Yokose*
*Department of Oncological Pathology, Cancer Center, Nara Medical College and **Department of Biochemistry, Shizuoka College of Pharmacy

Methylnaphthalene is a complex of α- and ß-isomer and exists as a contaminant in combustion gas of kerosine. We found that endogenous lipid pneumonia was induced by painting methylnaphthalene twice weekly on shaved skin on the backs of female $B_6C_3F_1$ mice. Endogenous lipid pneumonia developed in 3/11 mice (27%) and 16/16 mice (100%) given 59.7 mg/kg body weight methylnaphthalene for 61 and 105 weeks, respectively, and in 31/32 mice (97%) given 118.8 mg/kg for 61 weeks. Serial killing study showed that the requirement of times and doses for 100% induction of the pneumonia was 30 weeks and a total of 7128 mg methylnaphthalene in mice given 118.8 mg/kg and 20 weeks and a total of 9504 mg in mice given 237.6 mg/kg body weight.

Histopathologically, the pneumonia was characterized by swelling and proliferation of alveolar type-II cells, foam cell accumulation, the appearance of cholesterol crystals and thickening of alveolar interstitial spaces. Electronmicroscopically, the number and size of lamellar bodies in swollen and proliferating type-II cells were increased and foam cells contained degenerated lamellar bodies. Free lamellar bodies in the alveoli were also observed. Biochemically, cholesterol and dipalmitoylphosphatidylcholin and minor phospholipid increased in the lung with the pneumonia. The phospholipid was purified and identified as phosphatidylglycerol.

These results give important information for understanding the underlying mechanisms of endogenous lipid pneumonia in mice.

MODIFICATION OF LUNG TUMOR GROWTH BY HYPEROXIA - R.C. Lindenschmidt* and H.P. Witschi; Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831. *Present address: Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH 45247

Recent work in this laboratory showed that continuous exposure of mice to an atmosphere of 70% oxygen inhibited the development of lung tumors initiated by a pulmonary carcinogen. In the present work, the effects of hyperoxia on lung tumor development were examined further in mice and also in a second species, rats. Male A/J mice were injected intraperitoneally with various doses of the carcinogen urethan or 3-methylcholanthrene (3-MCA) and 4 days later placed into chambers containing either 21% O_2 (air control) or 70% O_2 until sacrifice 4 months later. Exposure to 70% O_2 significantly inhibited the development of urethan or 3-MCA-induced lung tumors at all dose levels. A potential mechanism of action of hyperoxia on lung tumors, interference in DNA synthesis, was investigated using *in vivo* autoradiography. Oxygen (70%) did not inhibit the incorporation of thymidine into the DNA of mouse lung tumor cells. Hyperoxia and tumor inhibition studies were conducted in a second species. Male F344 rats were treated with a single intratracheal instillation of 3-MCA and 1 week later were exposed to air, 40% or 70% O_2 for 7 weeks. Rats kept in air developed multiple lung lesions identified as keratinized squamous carcinomas. Hyperoxia (both 40% or 70% O_2) significantly reduced both tumor multiplicity and tumor incidence. No differences were found in the histologic appearance of the tumors in the treated and control rats. It is concluded that hyperoxia can inhibit development of transformed cells *in vivo* in the lungs of two species, mice and rats. It appears that this inhibition is not the result of interference with DNA synthesis. (Oper. by the Martin Marietta Energy Systems, Inc. with USDOE. RCL supported by the post graduate research training program administered by Oak Ridge Associated Universities for the U.S. Department of Energy.)

EFFECT OF A "NUISANCE" DUST INHALATION ON IMMUNOLOGIC FUNCTIONS OF RAT ALVEOLAR MACROPHAGES - M.-L. Lohmann-Matthes and H. Muhle, Fraunhofer-Institut für Toxikologie und Aerosolforschung, Hannover, FRG.

Particles for which no specific toxic effects are known are classified as "nuisance" dusts. A general threshold limit value has been established for an occupational exposure.
Test material were titanium dioxide, polyvinyl chloride powder (PVC) and iron powder at aerosol concentrations of 3, 8 and 20 mg/m^3. The exposure lasted up to 8 months. Alveolar macrophages from rats exposed to either dust or control air were tested for the following parameters: Production of tumor necrosis factor, spontaneous and activated extracellular cytotoxicity against tumor targets, spontaneous and activated intracellular cytotoxicity against Leishmania microorganisms. High concentrations of PVC exposure resulted in a preactivation of alveolar macrophages documented in all assay systems. In addition an elevated susceptibility for further activation was consistently observed with these macrophages.
In contrast high dosage exposure of iron powder resulted in a significant suppression of macrophages functions which was most pronounced in the extracellular tumor cell killing. The relevance of these results will be discussed.

CARCINOGENICITY OF DIESEL EXHAUST INHALED CHRONICALLY BY RATS - J.L. Mauderly, R.K. Jones, W.C. Griffith, R.F. Henderson and R.O. McClellan, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87185.

The finding of mutagenic activity in diesel exhaust soot led to concern for potential carcinogenesis in man. A chronic inhalation bioassay was performed in the rat to determine if diesel exhaust could be a pulmonary carcinogen. Male and female F344/Crl rats were exposed 7 hr/day, 5 day/wk for up to 30 mo, beginning at 4 mo of age to automotive diesel engine exhaust at soot concentrations of 0.35, 3.5 or 7.0 mg/m^3 or sham exposed to clean air. Exhaust was generated by 1980 5.7 L Oldsmobile engines operated on the Federal Test Procedure Cycle and burning certification fuel. Rats were sacrificed at 6 mo intervals to measure lung burdens of diesel soot and for histopathology. Other rats either died or were sacrificed after 30 mo of exposure. Lungs were fixed, sectioned into 3 mm slices and examined by dissecting microscope to detect tumors. Lesions were stained and examined by light microscopy. Survival and body weight were unaffected by exposure. Exhaust exposure did not affect body weight or survival. Other than soot accumulation, no adverse health effects were observed at the lowest exposure level. Focal fibrotic and proliferative lung disease accompanied a progressive accumulation of soot in the lung at the two highest exposure levels. After 24 mo of exposure, 20.8, 11.5 and 0.6 mg of soot were present in lungs of rats at the high, medium and low exposure levels, respectively. The prevalence of lung tumors was significantly increased at the high (13%) and medium (4%) dose levels above the control prevalence (1%). Four tumor types, all of epithelial origin, were observed: adenoma; adenocarcinoma; squamous cyst; and squamous cell carcinoma. Tumors occurred late during the exposures; 81% were observed after 24 mo. Logistic regression modeling demonstrated a significant relationship between tumor prevalence and both exposure concentration and soot lung burden. These results demonstrate that diesel exhaust, inhaled chronically at a high concentration, is a pulmonary carcinogen in the rat. (Research supported by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract No. DE-AC04-76EV01013.)

RELATIVE EFFECTS OF INHALED NITROGEN DIOXIDE AND DIESEL EXHAUST IN ADULT RATS AND RATS WITH DEVELOPING LUNGS - J.L. Mauderly, D.E. Bice, N.A. Gillett, R.F. Henderson, J.A. Pickrell and R.K. Wolff, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87185.

There is concern that infants and children might be more sensitive than adults to the effects of inhaled toxicants. In this study, the pulmonary effects of inhaled nitrogen dioxide (NO_2) and diesel exhaust (DE) in rats exposed as adults were compared to effects in rats exposed during lung development. Rats were exposed 7 hrs/day, 5 days/wk for 6 mo to NO_2 at 9.5 ppm, DE at 3.5 mg/m^3 soot, or to clean air as controls. Two age groups were studied; rats exposed beginning at 6 mo of age (adults) and rats exposed beginning at birth (young). Evaluations after the 6 mo exposure included respiratory function, airway fluid enzymes and cytology, pulmonary immune responses, lung tissue collagen and proteinases, histopathology, clearance of radiolabeled particles, and lung burdens of DE soot. NO_2 reduced body weight and altered airway fluids of both groups, but increased lung weight only in adults. DE altered airway fluid parameters and lung collagen and proteinases of both groups, but increased the number of cells in pulmonary lymph nodes and slowed particle clearance only adults. Lung burdens of soot were similar in both groups at the end of exposure, but the distribution of soot differed. Soot-laden alveolar macrophages formed focal aggregates in adults, but were scattered in young rats. Soot cleared from the lung more rapidly after the end of exposure in young rats than in adults. These results indicate that rats are not more sensitive to inhaled NO_2 or DE during lung development than during adulthood. These findings also suggest that the response of macrophages to inhaled soot particles is different in adult and developing rat lungs. The results of this study suggest that there might not be a need to consider infants and children as a special risk group for inhaled environmental pollutants.
(Research performed in facilities provided by the Office of Health and Environmental Research, U.S. Department of Energy with funds provided under Agreement 83-13-2 with The Health Effects Institute.)

EARLY DISTRIBUTION OF AN INHALED RADIOAEROSOL IN RATS - A.N. Payne, I.W. Lees, M.J.S. Gazeley, P.M. Webbon & G.E. Woolley, Dept. of Mediator Pharmacology, Wellcome Research Laboratories, Beckenham, Kent. BR3 3BS; Central Analytical Laboratories, (Biological), The Wellcome Foundation Limited, Dartford, Kent. DA1 5AH; and Depts. of Medicine, and Surgery, The Royal Veterinary College Field Site, North Mymms, Hatfield, Herts. AL9 7TA

We have used a simple and generally applicable isotopic method to compare the relative anatomical deposition and early distribution of aerosolised particles following head-only exposure of either conscious or anaesthetised rats.

Conscious or anaesthetised (Inactin 75mg kg^{-1} i.p.) male Wistar strain rats (235-265g) were exposed for 10 min to a radioaerosol of distilled water containing a tracer amount (0.3 mCi ml^{-1}) of ^{99m}Technicium pertechnate. The aerosol was generated by a Turnet jet-nebuliser driven by compressed air (14 p.s.i., flow rate 4L min^{-1}) delivering approximately 0.2 ml min^{-1}. The aerosol particle size distribution ranged from 0.7 - 15µm (mode 0.9µm, weight mean diameter of 9µm). At the end of the exposure period each animal was sacrificed with CO_2. The head, thorax and stomach regions were scanned for radioemission. Further readings were also taken from dissected tissues. (Table 1).

Table 1 Regional γ-radioemission (c sec^{-1}), energy peak 139-140 KeV.

Field/Tissue	Conscious	% Total	Anaesthetised	% Total
Head	1116 ± 206	36.8	724 ± 53	73.6
Thorax	380 ± 73	12.8	135 ± 26	13.8
Trachea	32 ± 15	1.1	15 ± 9	..6
Lungs	134 ± 44	4.5	60 ± 9	6.2
Oesophagus	135 ± 18	4.5	5 ± 2	0.6
Stomach -	1169 ± 621	38.6	26 ± 19	3.0
Duodenum	29 ± 20	1.0	5 ± 1	0.5
Heart	28 ± 11	1.0	6 ± 2	0.7

All values represent the mean (± s.e. mean) result from 5 animals in each group.

In conscious animals, relatively little deposition and subsequent retention of radioaerosol mass occurred in the lower respiratory tract (LRT) compared with either the head region or the gastrointestinal tract (GIT). In anaesthetised animals, there was a very marked reduction in radiomission from the GIT coupled with an increase in the proportion of radioaerosol retained in the head region. The most obvious explanation for this is a depression of the swallowing reflex.

CHRONIC EFFECTS FOLLOWING LONG-TERM EXPOSURE TO GASOLINE ENGINE EXHAUST IN RODENTS - U. Heinrich, L. Peters, and B. Bellmann, Fraunhofer-Institut für Toxikologie und Aerosolforschung, Hannover FRG.

Long-term studies with diesel engine exhausts have shown several chronic-toxic effects mainly in the respiratory tract of the experimental animals. Nowadays, however, at least in Europe about 90 % of automobiles burn leaded gasoline without catalytic converters. It, therefore, also seemed essential to investigate the possible chronic-toxic effects of gasoline engine exhaust in a long-term animal inhalation experiment.
Rats and hamsters were exposed to gasoline engine exhaust for 19 hrs/day, 5 days/week for up to 2 years. The exhaust was generated by a VW engine running on the US-72 driving cycle. The engine was operated with a leaded European reference fuel. The exhaust was diluted with clean air in ratios of about 1:60 and 1:30. The mean lifetime of the animals was not affected by the exposure. Only the rats exposed to the less diluted exhaust showed a lower body weight gain than the controls. Wet and dry weights of the lungs and hearts of both species increased slightly. Lead content was enriched in blood and bone in correlation to that of the exposure atmospheres. Clinical-chemical, haematological, biochemical and cytological investigations primarily showed some CO-induced changes in both species, mainly in the 1:30 exposure groups. Some of the exposure-related effects appeared to be reversible 6 months after termination of the exposure. After two years of exposure the respiratory tract, being the main target organ in inhalation experiments, showed predominantly in the rats a deterioration of the alveolar lung clearance and altered mechanical lung functions such as increased airway resistance and decreased lung compliance.

THE ROLE OF COMPLEMENT IN THE EARLY PATHOGENESIS OF ASBESTOSIS - DB Warheit, LH Overby, and AR Brody. Du Pont-Haskell Lab , Newark, DE and NIEHS, North Carolina, USA.

Asbestosis is a lung disease clearly associated with occupational and environmental exposure to inhaled asbestos fibers. The pathogenesis of this disease has not been well established. Pulmonary macrophages have been proposed as the connecting link between fiber-induced inflammation and the develpment of pulmonary fibrosis.
In experimental studies using rats and mice, we have previously shown that pulmonary macrophages migrate to sites of asbestos deposition to form a component of an early lesion. Here we report that inhaled asbestos fibers activate a complement-dependent chemoattractant thus recruiting macrophages to sites of fiber deposition, and facilitating the earliest lesions of asbestosis. To support this conclusion, inhalation studies were carried out where normal and decomplemented (CVF-treated) rats as well as congenic strains of C5-normal ($C5^+$) and C5-deficient ($C5^-$) mice were exposed to chrysotile asbestos. In addition, in vitro studies using pulmonary macrophage chemotaxis as a bioassay for complement activation were utilized. Our data showed that incubation of asbestos fibers with rat serum or normal lavaged lung progeins produced a substantial chemotactic response ($p<0.01$). Moreover, proteins lavaged from the lungs of asbestos-exposed rats produced significant chemotactic activity when compared to sham-exposed controls ($p<0.01$). To investigate the role of complement in facilitating the macrophage recruitment response, $C5^+$ and $C5^-$ mice, as well as normal and CVF-treated (cobra venom factor) rats were exposed to chrysotile asbestos for brief periods and the macrophage response was quantified by scanning electron microscopy (SEM). The numbers of accumulated macrophages were significantly decreased in the $C5^-$ mice and CVF-treated rats ($p<0.01$). Time course studies showed that chemotactic factor elaboration preceded the macrophage migration response ($p<0.01$). SEM x-ray spectroscopy revealed that decreased percentages of macrophages from CVF-treated, exposed rats phagocytized fibers. We conclude that complement activation by the inhaled fibers plays an important role in the early development of asbestosis.

CELL-TO-CELL AND CELL-TO-FIBER INTERACTIONS IN ASBESTOS-INDUCED LUNG DISEASE - KE Pinkerton & RR Mercer, University of California, Davis, CA and Duke University, Durham, NC.

Although the mechanisms by which asbestos fibers cause disease are not known, it appears that certain interactions between pulmonary cells and fibers are important. Recent work suggests that the accumulation and retention of asbestos in the pulmonary interstitium plays a key role in the genesis of fibrosis. However, movement of fibers to the interstitium and those cells involved in the translocation and subsequent events leading to fibrosis are not well understood. To address these questions morphometric methods and computer-assisted three-dimensional imaging were used to visualize and quantify these phenomena in the lungs of rats exposed by inhalation to chrysotile asbestos for up to 12 months. Of particular interest were associations of epithelial and inflammatory cells with mesenchymal cells in the presence of asbestos fibers. Epithelial transport of fibers was found to be literally an extracellular event. Passage of fibers via membrane-lined channels through intact viable epithelial cells was noted with one end of the fiber in the airspace and the other end in the interstitium. Once fibers gained access to the interstitial compartment, an intense cellular response was elicited consisting of fibroblasts, monocytes and macrophages. Three dimensional imaging of interstitial cells demonstrated a close apposition of monocytes and macrophages to fibroblasts with numerous plasma membrane contacts between these cells. Increased collagen deposition was commonly noted surrounding these cell masses. Multiple cells acting upon a single fiber were common with portions of a fiber contained in an epithelial cell and interstitial macrophage or fibroblast. Macrophages were the only cells containing extracellular pockets in which portions of asbestos fibers had become coated with calcium phosphate deposits. These findings suggest that the epithelium plays an important role in the movement of respired fibers to the interstitium. The response of the interstitium to the presence of asbestos consists of numerous cell contacts between fibroblasts, macrophages and monocytes subsequent to collagen deposition. The importance of these cell-to-cell communications and cell-to fiber interactions are likely to be central to the genesis of asbestos-induced lung disease.

DEGENERATIVE, INFLAMMATORY, AND PROLIFERATIVE LESIONS OF THE NASAL MUCOSA OF RATS AND MICE EXPOSED BY CHRONIC INHALATION TO SELECTED ORGANIC CHEMICALS WITH POTENTIAL FOR HUMAN OCCUPATIONAL EXPOSURE - R.A. Renne, R.A. Miller, W.E. Giddens, Battelle Northwest Laboratories, Richland, WA 99352, G.A. Boorman and R.R. Maronpot, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Several industrially important organic chemicals were selected by the National Toxicology Program for carcinogenicity/toxicity assays by chronic inhalation exposure. Groups of 50 Fischer 344/N rats and B6C3F1 mice of each sex were exposed in inhalation chambers to one of two or three concentrations of the test chemical for 6 hours per day, five days per week for up to 103 weeks. Rats and mice developed a wide spectrum of lesions in the nasal mucosa in response to inhalation exposure to these chemicals. Inhalation of propylene oxide induced epithelial tumors, hyperplasia, squamous metaplasia, and chronic rhinitis in rats and primary vascular tumors in the nasal mucosa of mice. Propylene induced nasal epithelial hyperplasia and squamous metaplasia in rats. Methyl methacrylate induced rhinitis and olfactory epithelial degeneration in rats and mice. 1,2-epoxybutane induced nasal epithelial tumors in rats and epithelial hyperplasia, squamous metaplasia, degeneration, and inflammation in rats and mice. 1,3-butadiene induced chronic rhinitis, osseous and cartilaginous metaplasia, and olfactory epithelial degeneration in mice. The morphology of these induced lesions will be described, illustrated, and compared with nasal lesions reported by other investigators in rodent inhalation toxicity and carcinogenesis studies.

LONG-TERM INHALATION STUDIES ON EFFECT OF EXHAUST FROM HEAVY AND LIGHT DUTY DIESEL ENGINS ON F-344 RATS - Y.Takaki, S.Kitamura,N.Kuwabara and Y.Fukuda,Juntendo University School of Medicine,Hongo,Bunkyo-ku,Tokyo,Japan.

In order to observe the carcinogenesis of diesel emission, long-term inhalation studies were carried out on SPF F-344 rats(4 weeks of age) using a heavy-duty(11 liter) and light-duty(1.8 liter) diesel engin.Each exhaust gas was diluted to five levels concentration,and was supplied to each inhalation chamber in the barrier.A group of rats consisted of 120 males and 120 females for each level of exhaust gas concentration.Rats were sacrified at 6,12,18,24 and 30 months.In addition,an experiment for observing the influences of particle was carried out using diluted and filtered exhaust gas in which particles of exhaust had been removed.
Histopathological examination revealed during 30 months of inhalation,swelling and hyperplasia of type-II alveolar epithelial cells surrounding the particle,hyperplasia of bronchiolar epithelia toward peripheral air space.These changes were found in the lungs after 6 months exposure.The number of the foci and the extent were increased in relation to the duration of exposure and the exhaust gas concentration.The incidence of primary lung tumors including adenoma,carcinoma and other tumors was dependent on heavy-duty diesel engin.However,the incidence was independent on light-duty diesel engin.
In the study of the filtered exhaust gas,there was no difference of histological changes of the lung from those of the control group.
Leukemia,breast tumor and other tumors were not signficantly increased in the experimental group as well as the control group following 30 months inhalation.

HYALURONIC ACID IN BRONCHOALVEOLAR LAVAGE; A MARKER OF FIBROTIC DEVELOPMENT IN QUARTZ-EXPOSED RATS? - G. Tornling, A. Eklund, G. Unge and R. Hernbrand, Department of Thoracic Medicine and Department of Clinical Chemistry, Karolinska Hospital, S-104 01 Stockholm, Sweden.

The level of hyaluronic acid (HA), a connective tissue component, in bronchoalveolar lavage fluid (BAL) is correlated to decreased lung volumes in sarcoidosis. In order to investigate whether hyaluronic acid could be a marker of fibrosis in another interstitial lung disease, silicosis, we measured the level of HA in BAL fluid from rats exposed to silica by intratracheal instillation. Three animals were exposed to 40 mg crystalline silica containing 98 % quartz with a mean diameter of 1.2 um. Three animals were exposed to 40 mg amorphous silicon dioxide with crystalline components less than 1 % and with a mean diameter of 0.05 um. One rat was sham injected with physiological saline. The total number of recovered alveolar cells was increased in the rats exposed to crystalline silica. There was a marked increase in the proportions of neutrophils and lymphocytes. The level of HA was higher in the quartz-exposed rats (mean 54 ug/l) than in the animals exposed to amorphous silicon dioxide (mean 7 ug/l) and the sham injected rat (2 ug/l). Microscopic evaluation of the lungs revealed massive pulmonary fibrosis in the quartz-exposed rats but not in the other animals. Thus, the level of HA in BAL fluid in rats exposed to crystalline silica seems to be a possible marker of fibrotic development.

CATEGORY E
RISK ASSESSMENTS FOR AIRBORNE TOXICANTS
PAPERS

AGE-DEPENDENT CHANGES AND NON-NEOPLASTIC SURFACE PROTRUSIONS IN THE LARYNGEAL AND TRACHEAL EPITHELIUM OF THE SYRIAN GOLDEN HAMSTER (MESOCRICETUS AURATUS) - C. Bröckmeyer and U. Heinrich, Fraunhofer-Institut für Toxikologie und Aerosolforschung, Nikolai-Fuchs-Str. 1, 3000 Hannover 61, FRG

The interior of the larynx and trachea of 8, 12.5 and 17-month-old hamsters was examined using scanning electron microscopy. Some of the specimens were successively embedded in Epon and investigated in semithin sections by light microscopy. Between the 8th and 17th months of life, simple metaplasia and slow mucus cell hyperplasia occurred in the hamster tracheae. Whilst an increase in mucus cells occurred between the cartilaginous rings, simple metaplasia was conspicuous in the surrounding large patches of unciliated cells, which covered the tracheal cartilages in the young hamsters. Flat or dome-shaped cells with microvilli covered the glottis and supraglottis of the 8-month-old hamsters where ciliated cells were rare. The vocal cords were covered by flat cells with microfolds and microridges. The older hamsters showed the same form of surface differentiation in the false fold area also.

Although no tumors were present, three types of surface protrusion were visible: (1) slow projections of tracheal mucosa caused by submucosal calcifications; (2) variously formed aggregations of mucus which protruded from mucus glands; and (3) papillary hyperplasia of the vocal cord mucosa.